A Classification of New Hampshire's Natural Stream Communities
Quality Assurance Project Plan

20 October 2003

Prepared by

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Principal Investigator and Project QA Manager:

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Robert T. Eckert, Department of Natural Resources, UNH Date

Project Manager:

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Brian Frappier, Department of Natural Resource, UNH Date

Program Quality Assurance Coordinator:

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Andrea Donlon, NHDES Date

NHDES Quality Assurance Manager:

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Vincent Perelli, NHDES Date

USEPA Project Manager:

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Warren Howard, US EPA Region I Date

USEPA QA Representative:

_____

Patrice Svetaka Date

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A3. Distribution List

Table 1 presents a list of people who will receive the approved QAPP, the QAPP revisions, and any amendments.

Table 1. QAPP Distribution List

QAPP Recipient Name	*Project Role*	*Organization*	*Telephone number and Email address*
Brian Frappier	Project Manager	UNH Department of Natural Resources	603-862-1051 frappier@cisunix.unh.edu
Jeff Merriam	Chemical Lab Manager	UNH Water Quality Analysis Lab	603-862-2341 jeff.merriam@unh.edu
Andrea Donlon	Program QA Coordinator	NHDES Watershed Management Bureau	603-271-8862 adonlon@des.state.nh.us
Vincent Perelli	NHDES Quality Assurance Manager	NH DES Planning Unit	603-271-8989 vperelli@des.state.nh.us
Warren Howard	USEPA Project Manager	USEPA New England	617-918-1587 Howard.Warren@epamail.epa.gov
Patrice Svetaka	USEPA QA Representative	USEPA New England	617-918-8396 svetaka.pat@epa.gov

A4. Project/Task Organization

This is a research project that has received funding through a NHDES Nonpoint Source Local Initiatives Grant, which is funded by EPA through section 319 of the Clean Water Act. The principal investigator of the project is:

Robert Eckert, Professor of Environmental Conservation
Department of Natural Resources, University of New Hampshire, Durham, NH 03824

Table 2 lists the personnel involved with the project and their respective responsibilities.

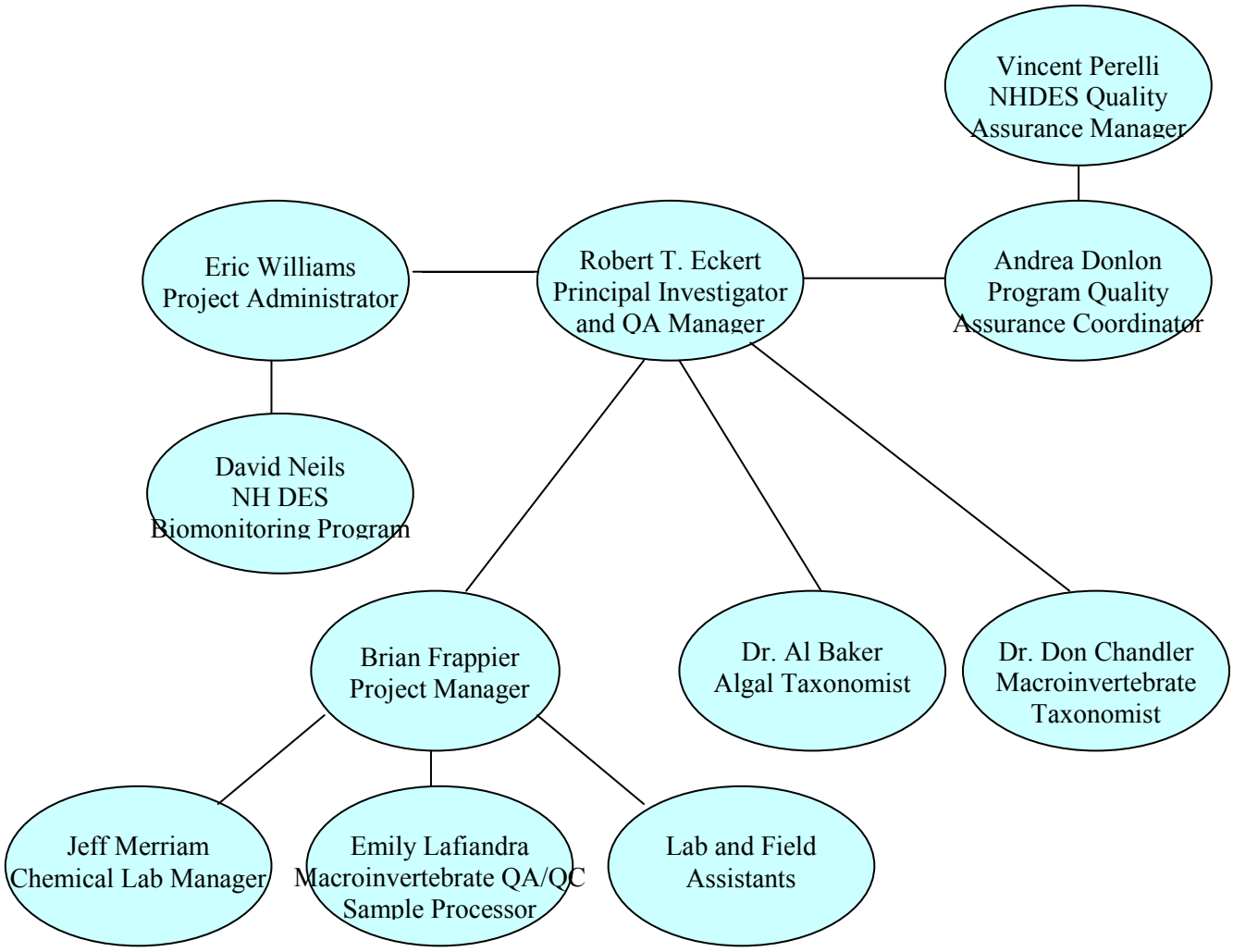
Table 2. Personnel Responsibilities and Qualifications

Name and Affiliation	*Responsibilities*	*Qualifications*

Name and Affiliation	*Responsibilities*	*Qualifications*
Robert T. Eckert Department of Natural Resources, UNH	Principal Investigator/QA manager; responsible for overall contract management, quality assurance objectives including analysis of reports from outside taxonomists, results of alkalinity QA objectives, and reports of data outliers in data accuracy screening; responsible for data analysis.	On file at DNR, UNH
Brian Frappier Department of Natural Resources, UNH	Project Manager; responsible for all field data collection including macroinvertebrates, periphyton, habitat variables, and water samples; responsible for all macroinvertebrate and periphyton sample processing, and data analysis.	On file at DNR, UNH
Jeff Merriam UNH Water Quality Analysis Laboratory	Chemical Lab Manager	On file at WQAL, UNH
Brian Topping Sarah Mikulak Danielle Adams	Field Assistants; assists with field activities under supervision of the Project Manager	On file with Project Manager
Meghan Motta	Biological Lab Assistant; responsible for macroinvertebrate sample sorting and data entry	On file with Project Manager
Emily LaFiandra Department of Natural Resources, UNH	Macroinvertebrate QA/QC Sample Processor; will re-identify 10% of identified macroinvertebrate samples	On file with Project Manager
Don Chandler Department of Zoology, UNH	Macroinvertebrate Systematist; will confirm macroinvertebrate voucher collection	On file at Department of Zoology, UNH
Al Baker Department of Plant Biology, UNH	Algae Systematist; will confirm algae voucher photographs	On file at Department of Plant Biology, UNH

Name and Affiliation	*Responsibilities*	*Qualifications*
Eric Williams NH DES Watershed Management Bureau	Project Administrator; reviews any changes to project, liaison between UNH and NH DES concerning administrative matters, receives semi-annual reports and final products.	On file at NHDES
David Neils NH DES Biomonitoring Program	Receives and reviews annual data products including quality assurance objectives reported by the QA manager; will be fully involved in data analysis and the development of reference site biocriteria from the total collected faunal and habitat data	On file at NHDES
Andrea Donlon NH DES Watershed Management Bureau	Reviews QAPP preparation and other QA/QC activities.	On file at NHDES

Figure 1. Flowchart of project personnel.



A5. Problem Definition/Background

This project will estimate the abundances of macroinvertebrate, fish, amphibian, and periphyton species and measure the co-occurring physical and chemical habitat factors of minimally impacted New Hampshire wadeable streams using nationally standardized sampling methods developed by USEPA-EMAP-SW (http://www.epa.gov/emap/html/pubs/docs/groupdocs/surfwatr/field/fomws.html) for field sampling and habitat description and USGS-NAWQA for biological laboratory methods (http://nwql.usgs.gov/Public/pubs/OFR00-212.html and http://water.usgs.gov/nawqa/protocols/algprotocol/index.html). Relative species abundances will be ordinated using Detrended Correspondence Analysis and axis scores will be related to environmental factors using stepwise multiple regressions. Natural communities will be classified based on relative species abundance using TWINSPAN, a complex divisive clustering method well accepted by community ecologists. Stepwise multiple discriminant analysis will be used to predict community type using physical and chemical habitat parameters. This investigation will provide the much-needed baseline data for further study into the basic patterns and processes of lotic ecology in the northeastern United States and provide quantitative biocriteria based on a minimally impacted reference conditions against which to assess the biological integrity of other lotic ecosystems in New Hampshire. Thus, the objectives are to:

- *Classify* the natural stream communities of New Hampshire and identify rare or unique communities
- *Discriminate between classified communities* using multiple scales of physical and chemical variables
- *Subdivide the aquatic ecoregions* in New Hampshire into subregions based on species assemblages
- Construct a model to *predict a theoretical reference community using physical variables* for biological monitoring and the setting of biological criteria
- Develop *a model to predict rare community locations in New Hampshire* and assess the level of representation of community types in conservation areas using physical landscape factors
- Provide *habitat models for endangered and threatened species* that are not sampled frequently enough

These data and analyses will potentially be used by the NHDES Biomonitoring Program to establish state-wide biological criteria for first to fourth order streams.

A6. Project/Task Description

This project will characterize the biota and physico-chemical habitat of 100-120 minimally impacted first to fourth order stream segments in New Hampshire. The specific methodology can be found in the appropriate sections of the QAPP. Because only a portion of the sites can be sampled and processed in any given year, this project

will involve four consecutive years of field sampling and sample processing. In each year of the project, sample sites will be identified, field sampling will be performed, samples will be processed in the lab, and quality assurance objectives will be assessed to ensure that the measurement performance criteria (as discussed in Section A7) are met. The same procedures will be performed each project year in the same order.

The resulting estimates of faunal and periphyton abundances will be ordinated and classified into biological community types using the divisive clustering technique TWINSPAN. Stepwise multiple discriminant analysis will be used to predict community type using the measured physical and chemical habitat parameters. Suggested biocriteria will be constructed using the range and mean abundance of each taxon found in the range of minimally impacted reference sites sampled in this project that are determined to contain a particular community type using the TWINSPAN analysis. In future bioassessment operations, these data and analyses could be used to predict the minimally impacted reference community that would inhabit an unknown test stream in the absence of pollution. Deviations in the test sample, as defined by NHDES in regulations, from the mean abundances of the predicted community type would indicate potential pollution impacts.

Data analysis and a final report will be produced in the final year of the project. The approximate dates for all activities can be found in Table 3.

Table 3. Project Schedule Timeline

Activity	*Anticipated Date(s) of Initiation*	*Anticipated Date(s) of Completion*	*Product*
QAPP Preparation	06/01/2002	05/30/2003	QAPP document
Reference site identification and screening	06/01/2002	06/01/2004	Site maps
Site sampling	06/01/2002	09/15/2005	Field data record sheets
Laboratory sample processing	09/16/2002	12/20/2005	Sample processing record sheets
Data validation	05/01/2003	01/15/2006	QA/QC record sheets
Data analysis	01/16/2006	01/30/2006	Statistical results and electronic data summary spreadsheets
Annual progress report	05/01/2003	06/01/2003	Annual progress report including quality assurance performance
Semi-annual progress report	11/15/2003	12/30/2003	Semi-annual report of progress since last

			report
Annual progress report	05/01/2004	06/01/2004	Annual progress report including quality assurance performance
Semi-annual progress report	11/15/2004	12/30/2004	Semi-annual report of progress since last report
Annual progress report	05/01/2005	06/01/2005	Annual progress report including quality assurance performance
Semi-annual progress report	11/15/2005	12/30/2005	Semi-annual report of progress since last report
Final project report preparation	02/01/2006	04/01/2006	Draft final report
Revise final report	05/15/2006	06/30/2006	Final report

A7. Quality Objectives and Criteria

New Hampshire Department of Environmental Services (NHDES) Biomonitoring program has documented generic quality assurance objectives for all biomonitoring projects in New Hampshire. Because the sampling techniques and biocriteria establishment approach in this project substantially differ from those used by NHDES Biomonitoring program, those generic quality assurance objectives are not appropriate for evaluating the performance of this project's goals and tasks. NHDES is aware that the quality assurance objectives in this document will deviate from the generic quality assurance objectives set for biomonitoring in New Hampshire.

Table 4 summarizes the performance criteria for samples collected for this project. The field and laboratory methods used in this study are based on standard methods for biological monitoring developed by the USEPA-EMAP-SW and USGS-NAWQA programs. The quality assurance performance criteria used in this project are the suggested performance criteria for those programs. The resulting data will be comparable to all NAWQA and EMAP-SW assessment programs. In addition, voucher collections and processed samples will be made immediately available for distribution to independent experts upon request.

Table 4. Measurement Performance Criteria for Biotic Surface Water Samples

Data Quality Indicators	*Measurement Performance Criteria*	*QC Sample and/or Activity Used to Assess Measurement Performance*
Precision-macroinvertebrate sorting	Number missed in sorted detritus < 10% of original number sorted	Repeat sorting of sorted detritus for 10% of samples
Precision-macroinvertebrate identification	Deviation from original count for each taxa < 10% of original count for that taxa in a sample	Duplicate enumeration and identification of the sorted macroinvertebrates for 10% of the samples by the macroinvertebrate QA/QC sample processor; Voucher collection identification by expert
Precision-periphyton identification	Deviation of percent community similarity from original count < 25%	Duplicate enumeration and identification of a new aliquot for 10% of samples by the Project Manager
Accuracy/Bias – macroinvertebrate and periphyton identification	Same procedures as for precision	Same procedures as for precision
Representativeness	Other studies have found that 10-15 sites per community type are necessary. We suspect that 4-7 community types will be present in NH.	A community type with less than 10 sites will be evaluated for potential clustering into the most similar type. Additionally, we will be using standard and well-documented field procedures, and training the individuals performing these activities
Comparability	Standard USEPA-EMAP-SW and USGS-NAWQA methods	Not deemed necessary

Sensitivity	As nothing is being compared nor hypotheses tested, this is not expected to be an issue for this project	---
Data Completeness	95% samples collected	Data Completeness Check

Table 5. Measurement Performance Criteria for Chemical Surface Water Samples

Data Quality Indicators	*Measurement Performance Criteria*	*QC Sample and/or Activity Used to Assess Measurement Performance*
Field Precision-Color Change Titration Alkalinity	15% relative percent difference from field duplicates	Duplicate collected every 25 samples
Laboratory Precision-Color Change Titration Alkalinity	10% relative percent difference from laboratory duplicates	Duplicate measured every 25 samples
Accuracy/Bias-Color Change Titration Alkalinity	10% relative percent difference from true value of Quality Control Samples (from Ultra Scientific)	5 replicate quality control sample titrations
Comparability	This is a standard method for determining Alkalinity	Not deemed necessary
Sensitivity	20 mg/L	---
Representativeness	None	None
Data Completeness	95% samples collected	Data Completeness Check

Table 6. Measurement Performance Criteria for *in-situ* Chemical Surface Water Measurements Using an Oakton 35630 Portable pH, Conductivity, and Temperature Meter.

Parameter	Meas. Range	Precision	Accuracy	Reporting Limit
pH	0.00 to 14.00 pH	± 0.01 pH	± 0.01 pH	---
Conductivity	0 to 19.99 µS 0 to 199.9 µS 0 to 1999 µS	± 0.01 µS ± 0.10 µS ± 1.00 µS	± 1%	0.01 µS
Temperature	0.0 to 100.0ºC	± 0.1ºC	± 0.5ºC	---

A8. Special Training/Certification

The Project Manager is experienced in fish, macroinvertebrate, and algae identification and general stream sampling techniques. No additional training is needed for the Project Manager. The Project Manager will train Field personnel at the beginning of each summer in correct techniques for obtaining quantitative physical data necessary to describe each sample site location. The training site will be listed as "PRACTICE" in the Site ID on the field forms and will not be included in the project data set or final data analysis. In addition, a field manual containing an outline of appropriate techniques and safety information will be available for reference in the field during all sampling efforts (Appendix D). Training logs for field and lab assistants will be recorded and kept with the project files.

A9. Documents and Records

Field and lab data sheets (see appendices A-C) will be on file with the Project Director for the project duration. After the internal data quality checking is complete, a summary data file will be given to NHDES on an annual basis as an Excel spreadsheet. Sites will be listed in rows and variables and other information in columns. The final data set will contain information about field-collected variables, taxa and their abundances in each sample, and information about any sample discrepancies (e.g. poorly preserved or damaged organisms; organisms not classified to desired levels and reason).

A final report summarizing all data and analyses will be provided to NHDES and USEPA by June 30 of 2006. In addition, electronic (and paper, if requested) files containing data on benthic macroinvertebrate, lotic vertebrate, periphyton, and physical and chemical samples collected each year will be provided to NHDES on an annual basis. The deadline for receipt of the annual data will be June 1 of each year. A semi-annual progress report detailing progress in data collection, processing, and analysis will be provided to NHDES each year the project is active. Quality Assurance and Performance Objectives measures will be included in the semi-annual reports as available.

At the conclusion of the project, the final data set will be provided to NHDES and USEPA as an Excel spreadsheet. Paper records and sorted macroinvertebrate samples will be archived by the Principal Investigator at the University of New Hampshire for the duration of his tenure. Following retirement, all paper records and macroinvertebrate samples will be given to NHDES for subsequent archiving.

The Principal Investigator, as the Quality Assurance Manager, will coordinate the dissemination of revised QAPP documents to all individuals on the QAPP distribution list (Table 1). Paper copies of the complete QAPP will be mailed after the approval of any change in policy or measurements.

B1. Sampling Process Design

Reference Site selection

The basic sampling unit will be a stream reach with a length 40 times the average wetted width of the stream near the sampling point. Stream segments, defined as the length of stream between two tributaries, will be randomly selected using the GRANIT hydrography GIS layer (Complex Systems Research Center 2001, http://www.granit.sr.unh.edu). A total of 100-120 segments will be sampled.

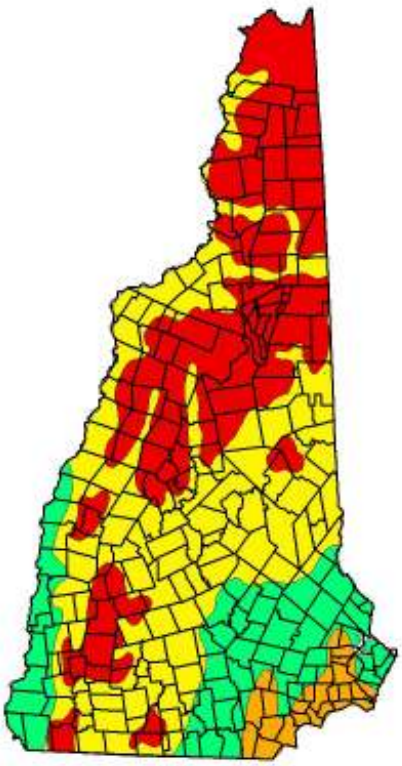
Selection of segments will be stratified by the stream length of each Level IV aquatic ecoregion (Omernik 1987, Figure 1). Ecological regions can be identified through the analysis of the patterns and the composition of biotic and abiotic phenomena that affect or reflect differences in ecosystems (Omernik 1987). These phenomena include geology, physiography, vegetation, climate, soils, land use, wildlife, and hydrology. The relative importance of each characteristic varies from one ecological region to another. Level I is the most coarse level, dividing North America into nine ecological regions. Level II, the continent, is subdivided into 32 classes. Level III further subdivides the continent into 78 classes. For portions of the United States, including New Hampshire, the ecoregions have been further subdivided to Level IV.

Each segment will be evaluated for anthropogenic impact using GIS and field visits. GIS layers identifying known point and non-point source pollution, land-use, right-of -ways, dams, public and private water extraction, and groundwater hazards were available from GRANIT. *Segments identified as having **any** upstream water quality threats using those layers will be discarded* and new segments selected until the required number of segments is reached. The length of roads and density of houses allowed in the upstream watershed will be determined through an iterative process identifying the minimal road length and housing density that can be achieved while also meeting the sampling goal of 100-120 reference sites. The final report will detail the definitions of "minimally impacted" that resulted from this iterative, exploratory process.

Site visits and professional judgment will be used to ensure additional threats not contained in GIS data are not readily apparent. The sampling reach will be randomly located along the segment, but at least 100 m upstream or downstream of the bounding tributaries and upstream of any roads or trails.

Figure 2. Level IV aquatic ecoregions of Omernik (1987) in New Hampshire

[Each color represents a separate Level IV ecoregion.]



B2. Sampling Methods Requirements

All sampling will be performed during the base flow period of 15 June to 15 September as recommended by USEPA-EMAP-SW (Peck et al. 2001, http://www.epa.gov/emap/html/pubs/docs/groupdocs/surfwatr/field/fomws.html). Sites that have experienced a recent spate will not be sampled for 6 weeks following the disturbance. It is difficult to define a spate for every part of New Hampshire. The flow profile that results from a large rainfall event depends on the amount and timing of rain and the degree of soil saturation due to previous weather. Thus, a spate will be determined by consideration of the above factors and National Weather Service reports of flood warnings. The Project Manager and Principal Investigator will reach a decision based on those parameters. The reasons for not sampling due to a spate as defined by those individuals will be documented.

The sample reach will be divided into 10 subsections delineated by 11 transects spanning the width of the stream. Each transect will be equally spaced along the reach (i.e. 4 wetted widths apart) with the first transect located at the downstream limit of the reach. A randomized, systematic spatial sampling design will be used to locate a sampling point on each transect according to the USEPA-EMAP-SW protocols. The

sampling point (left, center, or right) on the first transect will be randomly chosen. Subsequent sample locations will be assigned to each upstream transect, alternating in order as left, center, or right.

Habitat measurements – All habitat measurements will be made using the USEPA-EMAP-SW protocols (http://www.epa.gov/emap/html/pubs/docs/groupdocs/surfwatr/field/fomws.html). The following physical and chemical habitat variables will be measured at each sampling point: habitat type (pool, riffle, run), water depth, presence of soft/small sediment, and substrate size class (11 categories. A portable combined meter will be used to measure water temperature, dissolved oxygen, and pH at each sampling point. A digital canopy photo will also be taken.

The following physical habitat variables will be measured along each transect: Wetted width, intercept length and diameter of large woody debris, bank angle, undercut distance, bankfull channel width, aspect, canopy angles, bank height, flow velocity, embeddedness and size of 10 large substrate particles, and transect intercept distance of macrophyte cover.

For each reach, the water surface gradient between the upstream and downstream endpoints, altitude at the upstream and downstream points, and latitude and longitude of reach center will also be measured.

Lotic Vertebrates– Lotic vertebrates will be sampled using a Smith-Root LR24 Backpack Electrofisher after all other data collection and per the methods developed by USEPA-EMAP-SW. A single-pass electro-fishing method attempting to fish all available cover in the entire reach will be used starting at the downstream limit of the reach. In contrast to the USEPA-EMAP-SW methods, only 2 people will electroshock. A consistent effort will be applied throughout the reach for 50 minutes. Block nets will be placed at the downstream and upstream limits of the sampling reach when the sample reach is a large continuous pool. Collected fish and amphibians will be placed in a bucket of water, identified, searched for abnormalities, and returned to the stream. A number of published regional keys will be used for identification of individuals. Fish and amphibians of questionable taxonomy after reference to the keys will be killed and preserved in 70% ethanol for lab identification.

Periphyton - Periphyton will be collected at each sampling point using the methods developed by USEPA-EMAP-SW (http://www.epa.gov/emap/html/pubs/docs/groupdocs/surfwatr/field/fomws.html). Samples will be collected after water chemistry sample and before macroinvertebrate collection. In erosional habitats, a sample of rock or wood substrate will be removed from the stream. A 12 cm^2 area on the upper surface of the substrate will be brushed with a stiff-bristled toothbrush for 30 seconds to dislodge periphyton. Dislodged periphyton will be washed into a 500-ml bottle using stream water. In depositional habitats, the top 1 cm from a 12 cm^2 area of soft sediment will be vacuumed into a 60-ml syringe. Samples from the two habitat types will be compiled into a composite sample.

Macroinvertebrates – Macroinvertebrates samples will be collected at each of the transect sample points for a total of 11 samples per stream segment after periphyton collection. The USEPA-EMAP-SW benthic macroinvertebrate protocol will be used to collect quantitative macroinvertebrate samples in wadeable and non-wadeable streams

(http://www.epa.gov/emap/html/pubs/docs/groupdocs/surfwatr/field/fomws.html). A 500 µm modified d-net (net) will be used to collect organisms in all wadeable habitats.

In wadeable riffle or run habitats, the net will be placed securely on the stream bottom. Heavy organisms in a 0.5 m^2 sample area in front of the net will be hand-picked and placed into the net. A 20 second kick sample of the 0.5 m^2 sample area will be taken. At the end of the 20-second period, any organisms found on rocks in the sample area will be placed in the net. In wadeable pool habitats, heavy organisms in a 0.5 m^2 sample area will be hand-picked and placed into a net. The same 0.5 m^2 area of substrate will be disturbed by vigorous kicking. A 20-second sample will be collected by dragging the net repeatedly through the disturbed area just above the bottom while kicking. After kick sample is taken, organisms found on loose rocks in the sample area will be placed into the net. If the water is too shallow to use the net, the substrate will be stirred with gloved hands and a US Standard #30 sieve passes above the stirred area for 20 seconds. Net contents will be rinsed into a bucket half filled with water that will contain all of the samples as a single composite.

Water chemistry - Prior to any other sampling activities, water temperature, conductivity, and pH will be measured at the center point of the stream segment using an Oakton 35630 portable pH/conductivity/temperature meter. The meter will be operated and calibrated according to the procedures of the manufacturer (Appendix E) and Section B7. In addition, a 60 ml water sample will be taken at the downstream boundary of the reach for laboratory analysis of alkalinity. All samples will be filtered in the field through 0.7 um precombusted (5+ hours at 450 C) glass fiber filters (e.g. Whatman GF/F). Samples will be collected in acid-washed 60-mL HDPE bottles. Sample containers will be rinsed 3 times with filtered sample, and the bottle is filled with filtered sample. Samples will be stored in the dark and as cool as possible until they can be frozen. Samples must be frozen within 8 hours of sample collection. The sample will not be removed from the freezer until analysis by the Chemistry Lab Manager.

Sampling failures - The USEPA-EMAP-SW methods that form the basis for the sampling protocols have been tested and employed in a variety of streams (Peck et al. 2001, http://www.epa.gov/emap/html/pubs/docs/groupdocs/surfwatr/field/fomws.html). The protocols include specific and tested alterations of sampling approach in response to a variety of rare, but potential, sampling failures. In instances where a USEPA-EMAP-SW field method suggested change exists for a sampling failure situation, the suggested alterations in sampling will be made. If a situation occurs where normal sampling cannot be performed and no USEPA-EMAP-SW alternate protocol exists, then the project manager will decide on alternate methods. Any deviations will be recorded in the comments section of the appropriate field sheet (Appendix A). The Principal Investigator and Project Manager will discuss any deviations with NHDES to decide if the site should remain a part of the study or be excluded from any further analysis.

B3. Sample Handling and Custody Requirements

The sample site will be identified using the predetermined GRANIT ID label from the GRANIT hydrography GIS data layer for New Hampshire. The same Site ID will be used on all subsequent sample identification forms and sample vials to ensure unique

identification of all samples and data collection forms for the correct sample location. Data collection will be standardized using identical field forms for each sample locations (see Appendix A).

Macroinvertebrates and detritus collected in the kick-net samples will be transferred whole into one or two 1 L HDPE bottles and filled with 90% ethanol such that no bottle will contain more than 750 ml of sampled material. Benthic macroinvertebrate samples will be stored at the Principal Investigator's lab prior to lab analysis and sorting. A sample tracking form will record the date the lab received the sample and the dates of subsequent sorting and identification (Appendix B). Unsorted samples will be stored in their field collection containers in a metal file cabinet designated solely for this purpose. All sorted and counted specimens from each processed sample shall be archived in a box marked with the sample year and type of samples. Archived samples shall be preserved in 90% ethanol and vials labeled with the Site ID, number of vials, and number of sub-sampled grids inside the vial and on the outside. The Project Manager will maintain a reference collection (voucher collection) of all identified taxa.

Periphyton samples collected in the field will be transferred to 250ml HDPE bottles and preserved with formalin to achieve a 2% concentration in the sample bottle. The preserved samples will be stored at the Principal Investigator's lab prior to lab analysis and sorting. A sample tracking form will record the date the lab received the sample and the dates of subsequent sorting and identification (Appendix B). All identified subsamples shall be archived in a box marked with the sample year and type of samples. Archived samples shall be preserved in a container and medium appropriate to the taxonomic level used for identification. The archived subsamples will be labeled with Site ID and sub-sampling effort.

The water chemistry samples, stored as previously indicated in acid-washed 60 ml HDPE bottles, will be kept cool and dark while in the field. Each water chemistry sample will be identified with the Site ID and date collected on the outside of the sample container. The sample identifier will be recorded on the appropriate field form (Appendix A). Upon return to the lab, samples will be tracked using sample tracking forms (Appendix B). Samples must be frozen within 8 hours of sample collection. The sample will not be removed from the freezer until analysis by the Chemistry Lab Manager.

B4. Analytical Methods Requirements

Habitat data - USGS-NAWQA protocols and USGS BASINSOFT software (Meador et al. 1993) will be used to measure basin- (watershed-) level physical factors including drainage area, average annual runoff, average annual air temperature, average annual precipitation, average annual evaporation, basin length, minimum elevation, maximum elevation, basin relief ratio, drainage shape, stream length, cumulative perennial stream length, drainage density, drainage texture, entire stream gradient, estimated peak flow, flood volume, and seven-day low flow. Distance from source for each reach will be measured using the GRANIT hydrography GIS layer.

Macroinvertebrate sample processing – A fixed-count subsample procedure based on the USGS-NAWQA protocols (Moulton et al. 2000,

http://nwql.usgs.gov/Public/pubs/OFR00-212.html) will be used to estimate abundances of aquatic macroinvertebrates (Figure 1). Each sample will be rinsed and sieved using a 500 µm sieve. The sample will be uniformly distributed in a sub-sampling frame (stage-1 sub-sampling frame). An estimate of the average number of organisms per stage-1 grid will be obtained. Doberstein et al. (2000) found no significant differences in several taxa measurements between whole sample processing and 1000 count sample processing; however, lower count subsamples showed decreased power to detect differences between sites. Thus, a 500 fixed-count subsample will be used. An appropriate processing strategy will be selected based on the average number of organisms per stage-1 grid and the recommendations of Moulton et al. (2000). The grids will be randomly selected from either a stage-1 or a stage-2 sub-sampling frame, and organisms will be sorted from each grid. Large-rare organisms will be collected from any remaining unsorted portions of the sample.

A record of the processing will be kept on a standardized sub-sampling data sheet (Appendix C). The sorted debris will be retained in 90% ethanol and in the original field collection container for subsequent analysis of the efficiency of sorting by each sorter.

Samples will be identified to family by the Project Manager at the University of New Hampshire. Organisms that are obviously not benthic macroinvertebrates such as planktonic microcrustaceans (e.g., Daphnia), terrestrial species, and obvious "accidentals" shall be excluded.

These organisms, unsorted, will be preserved in a separate vial. A standard lab form repeating the pertinent site identification information will be used to enumerate and identify the benthic macroinvertebrates in each sample (Appendix C). Only one sample at a time will be identified.

Periphyton sample processing - Samples will be sub-sampled using the methods of USGS-NAWQA program (Charles et al. 2002, http://water.usgs.gov/nawqa/protocols/algprotocol/index.html). Analysis of relative abundance on the subsamples will be conducted using Palmer cell counts of 300 organisms by the strip count method. Because so little was known about New Hampshire lotic periphyton, the taxonomic level (e.g., morphology, class, or genus) that will be used has not yet been determined. After examination of several samples, recommendations will be made as to the level at which identifications should be performed. NHDES must approve of the taxonomic level used.

The Project Manager, a trained taxonomist, will perform all identifications. A standard lab form repeating the pertinent site identification information will be used to enumerate and identify the benthic macroinvertebrates in each sample (Appendix C). Only one sample at a time will be identified.

Digital photos of identified taxa will be collected from the microscopic field of view to be stored as a voucher collection in the Principal Investigator's lab.

Chemical analysis

The water sample for the reach will only be analyzed for alkalinity as this is relatively robust to most forms of anthropogenic disturbance and may be an important determinant of community structure (Wright 2000). Collected water will be analyzed for total alkalinity by the Chemistry Lab Manager, Water Resources Research Institute at the

University of New Hampshire, according to the standard color change titration method (Clesceri et al. 1989).

B5. Quality Control Requirements

Benthic Macroinvertebrate Performance Objectives

Sub-sampling efficiency - The first 3 samples each biological lab assistant sorts will be checked by the Project manager to ensure that at least 90% of the invertebrates were removed. Once a sorter has reached the target count, he/she will redistribute the sorted portion into a gridded sorting tray. The Project Manager will quality check the sample by resorting 20% (i.e. 3/15 grids) of the material according to the methods outlined above. The Project Manager will calculate an estimated percent efficacy by using the following equations:

a) Estimate the number of organisms missed:

$e = (a/b)c$

where:

e = estimated total number of organisms missed by sorter
a = the number of organisms found in the 20% resort
b = the number of grids resorted (usually 3)
c = the total number of grids in the gridded tray (usually 15)

b) Estimate the actual total count:

$c = a+b$

where:

c = the estimated total number of macroinvertebrates in the sorted portion of the original sample
a = the number of macroinvertebrates picked by the first sorter
b = the estimated number of macroinvertebrates missed (this is the value for "e" in equation #1)

c) Estimate the percent sorting efficacy:

$e = (a/b)100$

where:

e = the estimated percent sorting efficacy
a = number of macroinvertebrates picked by the first sorter
b = the estimated total number of macroinvertebrates (the value of "e" in equation #2)

The same process will be repeated by the Macroinvertebrate QA/QC Sample Processor for 10% of the completed samples. If the estimated percent sorting efficacy is 90% or greater the sample passes the QC check. If the estimate is less than 90%, the sorted portion of the original sample will be resorted. If this happens, the sample will undergo the QC process again until it passes 90% efficacy level.

Voucher collection – A voucher collection will be established consisting of at least one good specimen (preferably 3-5 specimens) of each taxon encountered. The voucher collections will be sent to the Macroinvertebrate Systematist for independent review. Samples that contain taxa that were found to be incorrectly identified by the expert review of the voucher collections will have those taxa re-identified.

Sample identification review – Ten percent of the samples will be re-identified by the Macroinvertebrate QA/QC Sample Processor. The individual samples to be checked are chosen at random after the samples are processed and the Project Manager will be unaware of which samples will be quality checked at the time of identification. The Macroinvertebrate QA/QC Sample Processor will review all records related to the sample to validate sample tracking.

The two taxonomists will discuss the results to determine what the taxonomic differences are as well as how to reconcile those differences. All discrepancies will be discussed and corrected as best as possible. Misidentifications will be corrected and errors in counts or data entry will be corrected as well. Enumeration of specific taxa may differ as a result of specimen loss and/or damage during repeated handling of the sample.

A percent similarity calculation will be used to compare the results. If results are consistently less than 90% similar and the Project Manager and Macroinvertebrate QA/QC Sample Processor cannot agree on their identifications, the Macroinvertebrate Systematist will be consulted to help clarify the problems.

Periphyton Performance Objectives

Sub-sampling error - The error associated with very small sub-sampling of a larger sample will be quantified by randomly selecting 3 samples to re-sample following initial sub-sampling. Each selected site will be sub-sampled four additional times. The replicate sub-sampling will be performed exactly as the usual sub-sampling techniques used for sample identification and enumeration. A mean and standard deviation of percent similarity will be calculated between the 5 replicates for each selected site. Replicate sub-samples should be at least 75% similar to each other as measured using an index of percent similarity.

Voucher collection - A voucher collection will be established consisting of one digital photo of each taxon encountered. The voucher collection will be sent to the Algae Systematist for independent review. Samples that contain taxa that were found to be incorrectly identified by the expert review of the voucher collections will be re-identified.

Water Chemistry - Alkalinity

Standards and reagents will be prepared from reagent grade chemicals (typically JT Baker) or from pre-made stock solutions. All glassware is acid washed (10% HCl) and rinsed 6 times with ultra pure-low DOC DI water (18.2 mega-ohm). A Laboratory Reagent Blank (LRB), Laboratory Fortified Blank (LFB) (a standard run as a sample) and Laboratory Duplicate will be analyzed every 20 samples during each run.

Quality Control Samples (QCS) (from Ultra Scientific) will be analyzed periodically (approximately every 20 samples) in each sample analysis batch to assure accuracy. The response/unit concentration is also used to monitor day-to-day variation in performance. A difference from the certified concentration of more than 10% is failure

and results in re-analysis of the entire sample queue, unless there is a very reasonable and supported explanation for the inconsistency.

Three QCS will be analyzed on each run. Duplicates of the QCS must fall within 10% relative percent difference (RPD = abs(dup1-dup2)/average of dup1 and dup 2). A difference greater than 10% is failure and results in re-analysis of the entire sample queue, unless there is a very reasonable and supported explanation for the inconsistency.

B6. Instrument/Equipment Testing, Inspection, and Maintenance

The Oakton portable water quality meter used during this investigation will be visually inspected prior to use for damage to the temperature probe and tested by comparing to standard solutions of pH and conductivity.

The current meter will be inspected prior to use and tested by conducting a "free spin" test to insure that the rotating current meter cups are free of obstruction and have freedom of movement during operation.

All nets and sieves will be examined for tears prior to sampling.

The Smith-Root backpack electrofisher will be visually inspected prior to sampling for proper electrical connections and tested for current using the audible alarms.

The alkalinity Quality Control Samples (from Ultra Scientific) will be inspected for damage and contamination prior to measuring.

B7. Instrument/Equipment Calibration and Frequency

The Oakton portable water quality meter will be calibrated in the laboratory according to the manufacturer's methods (Appendix E). All standard solutions used during the calibration process for the water quality meter will be previously unused, be within any expiration date, will be purchased from a reputable manufacturer, and will be specifically designed for this type of water quality meter.

Calibration of the water quality meter will be performed before every field sampling day. The pH probe will be calibrated using a three-point calibration utilizing commercial buffer solutions of pH 4.0, 7.0, and 10.0. The conductivity probe will be calibrated with a one-point calibration using commercial buffer solution of 100 µS. A one point calibration is used because each conductivity range on the Oakton 35630 Portable pH, Conductivity, and Temperature Meter uses a single calibration model calculated from a single calibration point (Appendix E). Stream conductivity in unimpacted New Hampshire streams are almost uniformly below 200 µS, thus wliminating the requirment for multiple calibration ranges . Temperature is factory calibrated and cannot be further calibrated by the operator.

B8. Inspection/Acceptance Requirements for Supplies and Consumables

The consumable supplies used in field sampling include 90% ethanol, 10% formalin, benthic sample bottles, algae sample bottles, acid washed water sample bottles, and glass fiber filters. The Project Manager will visually inspect all supplies before performing field sampling. If there is any evidence of contamination or damage, the supplies will not be used. Benthic sample bottles and algae sample bottles will be purchased new for the project. Water chemistry bottles will be acid washed as detailed in section B5.

The 90% ethanol, formalin, and calibration solutions used in the lab will be visually inspected by the Project Manager. Standards and reagents and Quality Control Samples for the alkalinity analyses will be visually inspected by the Chemical Lab Manager. If there is any evidence of contamination or damage, the supplies will not be used.

B9. Non-direct Measurements

The USGS software BASINSOFT will be used to calculate basin-level physical characteristics as identified in section B4. (Harvey and Eash, USGS fact sheet; http://gis.esri.com/library/userconf/proc96/TO100/PAP072/P72.HTM). All GIS coverages used to select sample stream segments and determine potential pollution sources are available through GRANIT, Complex Systems Research Center, University of New Hampshire (http://www.granit.sr.unh.edu/) in cooperation with the New Hampshire Office of State Planning and NHDES.

B10. Data Management

Data forms and data entry

Standard forms will be used to collect all field data and sample identification information (Appendix A). This will reduce error associated with data entry. Data reduction and validation are performed in an overall project spreadsheet (MS Excel). Outputs from the BASINSOFT software for each sampled basin also will be stored on the Project Manager's computer. The summary data for each sample site basin will be imported into the project spreadsheet by the Project Manager. Protocols, QC charts, and the project spreadsheet will be kept on the Project Manager's computer. These will be backed up weekly, with the back up stored off site. The computer is password protected, and is only used by the Project Manager. Handwritten sample processing sheets will be stored in a filing cabinet in the lab. All information pertinent to a sample is stored in the sample database.

Sample Storage and Tracking

Each sample will contain a unique sample identifier using the Site ID and all samples will be tracked using the appropriate sample tracking forms (Appendix B). Samples will be tracked from the time they are received, at each intermediate processing step, and at final archiving. The contractor will provide NHDES with an electronic sample tracking data file with the necessary sample tracking information and copies of the original sample tracking forms.

The contractor will be responsible for archiving the identified macroinvertebrate vials and the portion of the algae samples remaining after enumeration. These samples will be stored in a cabinet solely dedicated for that purpose according to the type of sample and the year collected. Samples will be preserved according to the techniques described in the previous sections.

C1. Assessments and Response Actions

The previously described quality performance objectives will be performed in an ongoing manner as data is collected and samples are processed. The Principal Investigator will be responsible for reviewing all performance objectives. Appendix C contains the lab forms used to document the QA assessments of the macroinvertebrate sample processing. The review of the reference collection by the pertinent macroinvertebrate and periphyton taxonomists listed in Table 2 will be documented in a letter from the taxonomist detailing any discrepancies in identification.

In cases where a specific corrective action has not been identified, the Principal Investigator, Project Manager, Project Administrator, and David Neils of the NHDES Biomonitoring Program will decide the most appropriate corrective action to be taken.

C2. Reports to Management

After the internal data quality checking is complete, a summary data file will be given to NHDES on an annual basis as an Excel spreadsheet. Sites will be listed in rows and variables and other information in columns. The final data set will contain information about field collected variables, taxa and their abundances in each sample, and information about any sample discrepancies (e.g. poorly preserved or damaged organisms; organisms not classified to desired levels and reason). The annual report will contain all quality objective results. The deadline for receipt of the annual data will be June 1 of each year.

A semi-annual progress report detailing progress in data collection, processing, and analysis will be provided to NHDES each year the project is active. Quality Assurance and Performance Objectives measures will be included in the semi-annual as available. This report will contain:

- A summary of precision, accuracy and completeness of all samples processed.
- A discussion of any problems that could affect the quality of the data along with a summary of corrective actions taken.
- Any changes (agreed to with USEPA prior to initiation) to the project protocols.

A final report summarizing all data, analyses, and quality objective results will be provided by June 31 of 2005. In addition, electronic (and paper, if requested) files containing data on benthic macroinvertebrate, lotic vertebrate, periphyton, and physical and chemical samples collected each year will be provided to New Hampshire Department of Environmental Services (NHDES) on an annual basis and to the USEPA at the conclusion of the project.

D1. Data Review, Validation, and Verification

The Project Manager will review the field forms at the end of each sampling day to ensure completeness and accuracy of data collection. Missing information will be collected before leaving the site if it is possible to accurately identify where it should have been taken.

Data entry into the master electronic spreadsheet by technicians will be double checked at random, but at least once every data entry session, by the Project Manager. If an error is found in an entry, it will be corrected and 3 more entries for that variable checked to assess if a systematic entry error has occurred. If the error is systematic, then all entries for that variable made by the lab assistant for that session will be corrected. In addition, outliers in the data set will be identified using SPSS (version 11.5) outlier identification routines. Standard statistical measurements appropriate to each data type (continuous, nominal, ordinal, etc.) will be used to assess outlier status of any data point.

D2. Validation and Verification Methods

Appendix C contains the lab forms used to document the QA assessments of the macroinvertebrate sample processing. The review of the reference collection by the pertinent macroinvertebrate and periphyton taxonomists listed in Table 2 will be documented in a letter from the taxonomist detailing any discrepancies in identification.

Errors found by the Project Manager while double-checking data entered by the Laboratory Assistant will be corrected. If an error is found in an entry, it will be corrected and 3 more entries for that variable checked to assess if a systematic entry error has occurred. If the error is systematic, then all entries for that variable made by the lab assistant for that session will be corrected.

Data points flagged as statistical outliers by a standard statistical analysis package will be re-examined by the Principal Investigator and QA Manager to ensure accurate recording from the field and lab bench sheets. Data points will only be changed if they disagree with the field data sheets.

D3. Reconciliation with User Requirements.

If the project objectives from Section A7 are met, the user requirements have been met. If the project objectives have not been met, corrective action as discussed in D2 will be established by the Project Manager prior to the next sample collection event.

David Neils of NHDES Biomonitoring Program will be directly involved with the building of suggested biocriteria using the numerical faunal and habitat data gathered from the reference sites sampled in this project. His comments, concerns and biomonitoring program requirements will be an integral part of the specific statistical data analysis procedures used to establish suggested biocriteria for wadeable streams in New Hampshire.

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APPENDIX A: Field Data Sheets

APPENDIX B: Sample Log Sheets

APPENDIX C: Sample Processing Sheets

APPENDIX D: Field Manual

APPENDIX E: Oakton pH/Conductivity/Temperature Meter Operation and Calibration Procedures